pressure is low in the advanced cases. In the early cases the blood-pressure picture, as a rule, is normal or generally decreased. If the heart is large naturally the blood-pressure picture takes on more the form of that in cases of enlarged heart with general depression of the blood-pressure picture. In the acute fevers the pressure picture in the initial stages shows no particular changes. As the cases develop the whole pressure picture as a rule shows a general depression and a low pulse-pressure usually means a badly diseased heart and is an unfavorable prognostic sign.

In general when the systolic pressure is normal, increased pulse-pressure signifies peripheral dilatation. When the systolic pressure is high the increased pulse-pressure is to be regarded as normal and has no special significance. Decreased pulse-pressure or even normal pulse-pressure means marked peripheral constriction, and is, therefore, an unfavorable prognostic sign.

With low systolic pressure increased pulse-pressure occurs in practically only one condition, aortic insufficiency.

With normal systolic pressure gradually decreasing pulse-pressure usually means cardiac failure. The condition is seen often in the later stages of the high systolic increased pulse-pressure cases.

With low systolic pressure decreased pulse-pressure usually means general cardiac weakness, as seen in febrile conditions or in convalescence from long illnesses or in cachexia of malignant disease.

A CENTRIFUGE METHOD TO PROVIDE A UNIFORM STANDARD FOR WASSERMANN READINGS.

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Immediately following its introduction as a diagnostic measure the Wassermann reaction was received with acclaim as a scientific test giving 100 per cent. average, but owing to the failure of laboratory reports to check up with the clinical findings in certain cases or owing to the failure of various laboratory workers to arrive at the same end reaction in a given case, when using the same specimen of serum, owing to laboratory reports remaining positive in various degrees longer than the clinician thought was commensurate with the amount of treatment, and many other such factors gradually led to a feeling of adverse criticism against the specificity of the Wassermann reaction, a feeling varying from a mere suspicion to an outspoken disbelief in its reliability as a laboratory procedure.

During this period there have been introduced various modifications of the original technique. Some of these modifications were designed to simplify the original method; others made a radical change in the character of the reaction, as in the substitution of the antihuman hemolytic system for the antisheep hemolytic system; another modification introduced the single unit system; again the so-called Baurer modification, and still others designed to decrease the amount of time necessary for the completion of the reaction, an important factor in the life of a busy laboratory.

When it became known that the reaction would occur in the presence of certain lipoidal extracts used as antigens, equally as well as with antigens prepared from syphilitic tissues containing Spirocheta pallida, a death-blow was thought to have been given to the specificity of the Wassermann test. Still later it was found that non-specific lipoidal extracts, to which was added cholesterin, became even more active in their complement-binding power. It has been urged[1] that such antigens were more delicate in detecting the presence of syphilitic antibodies and were therefore the antigens of choice to be used in following the therapeutic progress of a given case.

By other writers[2] it has been held that cholesterinized lipoids, granting that they were more sensitive in detecting syphilitic antibodies, nevertheless gave a certain number of non-specific reactions leading to the grave possibility of treating for syphilis a few or many individuals never afflicted with that disease. Clearly then it is better to test all serums against both syphilitic extract antigens and cholesterinized lipoidal antigens. We feel that for diagnostic purposes it is not safe to use only a cholesterinized lipoid as an antigen.

And so through persevering work and out of the knowledge born from experience in testing thousands of sera, one writer after another has called attention to and eliminated sources of error, until now it may safely be said of the Wassermann reaction, even though the original claims for specificity had to be abandoned, that for purposes of practical utility as a method of diagnosis and prognosis in syphilis this reaction now occupies a sound position, rarely excelled in laboratory procedures.

It need not be emphasized that absolutely reliable reagents should be used in the way of amboceptors, complements, antigens, etc., and that absolutely clean glassware, preferably of the Jena type, cleaned without any chemical, should be used. These things are generally checked up in all first-class laboratories.

In this paper we are not attempting any radical departure from the established Wassermann reaction technique, but are suggesting a simple modification which we believe will not only increase the accuracy of the test, but will also eliminate two sources of error, which will be discussed later.

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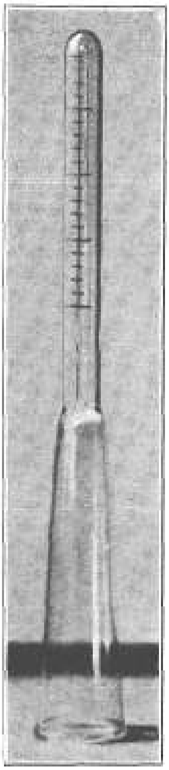
[1] John Kolmer, Pennsylvania Med. Jour., December, 1913; Lloyd Thompson, Jour. Am. Med. Assoc., May 9, 1914, xlii, 1458.

[2] B. A. Thomas and R. H. Ivy, Jour. Am. Med. Assoc., January 31, 1914, lxii, 363.

This modification in its fundamentals consists in the use of a graduated capillary centrifuge tube to which is transferred the contents of each Wassermann tube at the end of one hour's incubation and by centrifugation the amount of sheep's cells unhemolyzed in each serum reaction is determined. These special tubes are of the centrifuge type, having a total capacity of 4 c.c., and the tip of which is barometer tubing graduated in twenty divisions, each division or unit being equal to 0.0025 c.c.[3]



Graduated capillary centrifuge tube.

In order to make later discussion of the method more clear it seems best to introduce the protocol at this point.

PROTOCOL. *Reagents.* A. Treat sheep's blood by the usual method and make an emulsion of the thoroughly washed red cells in a 0.85 per cent. saline in a dilution 1 to 10 with a certified pipette graduated in hundredths; introduce into four centrifuge tubes 0.5 c.c. of this emulsion and add saline q. s. 2.5 c.c.

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[3] These tubes may be obtained through the Edward P. Dolbey Co., Philadelphia, Pa.

Centrifuge for five minutes (exactly) with a high-speed electrical centrifuge at not less than 1200 revolutions per minute. Read the amount of cells thrown down and average the amount of cells in the four tubes. This average should fall between 18 and 20 units. If not, adjust the emulsion so that it will.

B. Amboceptor prepared and standardized by the usual methods.

C. Complement. Fresh guinea-pig serum in dilution 1 to 10 saline.

D. Antigen. Prepared and standardized by usual methods.

E. Determine complement unit by titration as follows: Use eleven tubes and add to the first eight, properly labelled:

1. Increasing amount of complement (1 to 10) from 0.1 c.c. to 0.8 c.c.
2. 0.5 c.c. amboceptor representing 1 unit in proper dilution.
3. 0.2 c.c. (one unit) antigen.
4. 0.5 c.c. sheep cells 1 to 10.
5. To tube 9 add 0.5 c.c. sheep's cells, 1 to 10, and 1 unit of amboceptor.
6. To tube 10 add 0.5 c.c. sheep's cells, 1 to 10, and 1 unit of complement.
7. To tube 11 add 0.5 c.c. sheep's cells, 1 to 10.
8. To all tubes add normal saline q. s. 2.5 c.c.

Incubate for forty-five minutes in water-bath at 37.5° C. (Shake tubes thoroughly after ten- and twenty-minute incubation.)

Centrifuge contents of first eight tubes. Take for unit of complement the quantity used in the tube which shows 3 to 4 cell units of unhemolyzed cells.

Tubes 9, 10, and 11 should show a complete inhibition hemolysis.

It is not necessary, in our opinion, to titrate both the amboceptor and the complement units separately, for the two work in a definite combination in an inverse proportion, that is, as the complement unit is increased the amboceptor unit must be decreased, and *vice versa* (cf. Chart No. 1). (It is important, however, to adjust the dilution of the amboceptor so that the complement unit falls in the 0.4 c.c., 0.5 c.c., or 0.6 c.c. tubes, lower units than these being productive of disconcerting inhibition of hemolysis in the control tubes.)

F. *Test Set.* (Cf. Chart No. 2.)

I. Add to tubes in first row:

1. 0.1 c.c. suspected serum (inactivated at 55° C. for thirty minutes).
2. One unit of complement (as determined in E).
3. One unit of antigen. (N.B.—It is our custom to use at least two antigens, either of the same or different types.)
4. Normal saline q. s. 1.5 c.c.

Chart I.—Demonstrating in the Titration of the Complement that as the Complement Unit is Increased the Amboceptor unit must be Decreased, and Vice Versa. .5 c.c. Sheep's Cells, 1 to 10, = 18.7 Cell Units.

Tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Complement 1 to 10, c.c.	.1	.2	.3	.4	.5	.6	.1	.2	.3	.4	.5	.6	.1	.2	.3	.4	.5	.6
Amboceptor 1 to 1000, c.c.	.5	.5	.5	.5	.5	.5	.25	.25	.25	.25	.25	.25	.1	.1	.1	.1	.1	.1
Sheep's cells, 1 to 10, c.c.	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5
Saline									q. s. 2.5 c.c.									
						Incubated for 45 min. at 37.5° C. in water bath.												
Unhemolyzed cells, units	10.8	4.2	3.1	1.9	1.7	1.	13.8	8.5	5.9	5.9	5.9	3.6	16.9	14.9	11.8	11.8	11.8	9.0
Cells hemolyzed, units	7.9	14.5	15.6	16.8	17.	17.7	4.9	10.2	12.8	12.8	12.8	15.1	1.8	3.8	6.9	6.9	6.9	9.7

Chart II.—Protocol.

	Inactivated Patient's serum, c.c.	Complement unit determined by titration, units.	Antigen, units.	Sheep's cells 1 to 10, c.c.	Saline.		Amboceptor unit made up to 0.5 c.c.	Sheep's cells 1 to 10, c.c.	Saline.		
Tubes in I row	.1	1	1	0	q. s. 1.5 c.c.	Incubate for 45 minutes at 37.5° C. in water-bath.	X c.c.	.5	q. s. 2.5 c.c.	Incubate for 45 minutes at 37.5° C. in water-bath.	Centrifuge contents of all tubes for 5 minutes at X revolutions (*about* 1200 per minute). Record amount of unhemolyzed cells.
Tubes in II row	.1	1	0	0	q. s. 1.5 c.c.		X c.c.	.5	q. s. 2.5 c.c.		
Tubes in III row	.05	1	0	.25	q. s. 2.5 c.c.						
Alpha tubes	0	1	1	0	q. s. 2 c.c.		0	.5	0		
Beta tubes	0	1	0	0	q. s. 1.5 c.c.		.5	.5	0		
Reagents' control	0	1	1	0	q. s. 1.5 c.c.		.5	.5	0		

II. Add to the tubes in second row:

1. 0.1 c.c. suspected serum, inactivated.
2. One unit of complement.
3. Normal saline q. s. 1.5 c.c.

III. Add to tubes in third row:

1. 0.05 c.c. suspected serum.
2. One unit of complement.
3. 0.25 c.c. sheep cells 1 to 10.
4. Normal saline q. s. 2.5 c.c.

IV. Prepare a set of four tubes, called Alpha tubes, each tube to receive:

1. One unit of antigen.
2. One unit of complement.
3. Saline q. s. 1.5 c.c.

V. Prepare a set of four tubes called Beta tubes, each tube to receive:

1. One unit of complement.
2. Saline q. s. 1.5 c.c.

VI. As reagent control prepare one tube to contain:

1. One unit of complement.
2. One unit of antigen.
3. Saline q. s. 1.5 c.c.

Shake and incubate the foregoing tubes for forty-five minutes at 37.5° C. in a water-bath and then add to tubes under I and II:

1. 0.5 c.c. sheep's cell emulsion 1 to 10.
2. 0.5 c.c. (?) amboceptor = 1 unit except to all sera which in III row tubes show complete hemolysis, indicating presence of sufficient native sheep amboceptor. No artificial amboceptor is to be added to these sera. Sera which show partial hemolysis in III row tubes are to receive the proper fraction of the amboceptor unit.

To tubes in IV (Alpha tubes) add:

1. 0.5 c.c. sheep's cell emulsion 1 to 10.

To tubes in V (Beta tubes) and VI (reagent control tube) add:

1. 0.5 c.c. sheep emulsion 1 to 10.
2. 0.5 c.c. (?) amboceptor = one unit.

Shake and incubate all tubes (except those under III) for forty-five minutes in water-bath incubator at 37.5° C. Shake tubes again after ten minutes and twenty minutes' incubation.

G. Take readings as follows: Transfer by means of Wright's pipettes the entire contents of each of the foregoing tubes into the special centrifuge tubes, washing clean with a slight excess of normal saline:

1. Centrifugalize the Alpha tubes and average them.
2. Centrifugalize the Beta tubes and average them.

The average Alpha minus the average Beta will represent the average amount of cells hemolyzed in a negative reaction, or conversely the average number of units of unhemolyzed cells in a 100 per cent. positive serum.

3. Centrifugalize all the test and control tubes and establish the strength of reaction of each by a percentage scale. Record the readings in the following way:

Date May, 1914	No. of serum	Name	Unhemolyzed cells in Series I (front row) expressed in centrifuge units.	Unhemolyzed cells in Series II (back row) expressed in centrifuge units.	Unhemolyzed cells in front tube minus those in back or control tube.	100 per cent. for each serum is represented by alpha minus number of units in series II, or back tube.	Per cent. of cells not hemolyzed in suspected serum *i. e.*, percentage of positivity.
	1	A	10.8	2.4	8.4	16.4	51.2
	2	B	16.2	2.2	14.0	16.6	84.3
	3	C	11.2	4.0	7.2	14.8	48.6
	4	D	.5	.3	.2	18.5	0
	5	E	18.9	1.0	17.8	17.7	100

Alpha = 18.8 Beta = 2.8. Alpha minus Beta = 16 or an average of 100 per cent.

To determine the percentage of positiveness of each serum proceed as follows:

After centrifugation read the number of cells left unhemolyzed (to be hereafter called "cell units") in the front row tube and subtract from that the number of "cell units" in the back row tube—the control tube.

Now the average number of "cell units" of the four "Alpha tubes" minus the average "cell units" in the four Beta tubes will represent the average 100 per cent. positive for the entire reaction, the 100 per cent. here being equivalent to the absorption of one complement unit.

It is possible to establish an individual 100 per cent. for each serum in the reaction by subtracting the number of "cell units" in the serum control tube from the Alpha average. Therefore the actual 100 per cent. of each serum is easily calculated by comparing the difference in "cell units" of the serum tube and its control with the 100 per cent. established for that serum. It is quite essential to establish this 100 per cent. for each individual serum for two reasons: (1) because certain sera may contain native sheep amboceptor beyond the double unit content as determined by the Bauer modification, so that the number of "cell units" will fall below the Beta established for the reaction; (2) to eliminate errors introduced by slight anticomplementary action in certain sera in which the number of "cell units" in the control tube slightly exceeds the Beta established for the reaction.

Take, for example, that while 16 cell units represents the average 100 per cent., 14.8 cell units is the individual 100 per cent. for serum No. 3 (cf. Chart.) Now if 14.8 is 100 per cent. what is the percentage of 7.2? This is obtained by simple proportion as follows: 14.8 : 7.2 :: 100 : X.

Occasionaly strongly positive sera will be encountered whose unhemolyzed cell bulk may fall a few units below the cell bulk of the average Alpha without, however, showing any evidence of hemolysis. Such sera, though falling slightly below 100 per cent. on calculation (say 98 per cent.), should, nevertheless, be considered 100 per cent. reactions, *provided* no visible hemolysis has taken place. At present we are unable to explain this phenomenon, except possibly on a theory of Kaplan regarding surface tension relations

It will be seen by studying the protocol that in this proposed modification there is little difference from the Wassermann technique generally in use, except in the fact that at the end of forty-five minutes' water-bath incubation the material is transferred from the Wassermann tube to the centrifuge tube and after centrifugation the final reaction is read *at once,* and except that we insist upon having 3 to 4 cell units unhemolyzed in the control tubes (as already determined by the complement titration), so that we may be sure that hemolysis has proceeded as far as it can within the given limit of time. As long as this principle is adhered to the exact reading of each individual serum is under accurate control. Otherwise the technique is the one unit system, which to our view appears the most rational. We also include a tube to detect the presence, in each serum, of native sheep amboceptor, sufficient in itself to cause complete hemolysis.[4]

We agree with other writers[5] that the presence of sufficient native sheep amboceptor occurs rather more frequently than the serologists who do not include this check on the serum are aware of. This also creates an error in diagnosis to the negative side.

Where the presence of native sheep amboceptor was not checked we frequently observed that in some sera the total amount of cells contained in the control tube was completely hemolyzed, while the Beta average was perhaps 4.2 units, suggesting that the increased hemolysis came from the excess amboceptor—and if this hemolysis occurred in the back or control tube it must likewise occur in the front or diagnosis tube.

In a similar way we may infer the presence of anticomplementary bodies in the serum when the cell units unhemolyzed in back or control tube greatly exceeds the average determined by the Beta tubes—where this occurs in sera, for diagnosis, we think it unsafe to interpret the strength of the reaction by subtracting the back tube unit from the front tube unit and prefer to request a fresh specimen of serum. That it is unsafe to interpret such sera has forced itself on us, because it has been our custom to transfer positive and negative sera from the German Hospital, where the reactions are done on Wednesdays, to be used as controls in the reactions performed at the Methodist Hospital on Saturdays, and *vice versa*, thinking that such sera would be fresher as controls after three days' refrigeration than if kept for one full week in the ice-box. The same technique and the same reagents are used at both hospitals by the same laboratory worker, and yet it occurs, not infrequently, that sera that were strongly positive at the German Hospital, when tested again as a control, three days later, had developed sufficient anticomplementary reaction as to cause 50 per cent. inhibition of hemolysis in the control tube. Therefore it is of the utmost importance to test sera within twenty-four to forty-eight hours after withdrawing the blood.

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[4] J. Bauer, Deutsch. med. Wochnschr., 1908, xxxiv, 698

[5] Dexter and Cummer, Arch. Int. Med., May, 1912, ix, 605.

It will be noted that the final reactions by this method are made at the end of forty-five minutes' incubation when the transfer of contents from Wassermann to centrifuge tubes is made. This is to eliminate the first of the two errors referred to earlier in the paper.

This first source of error lies in the following fact: In titrating for either the complement or the amboceptor unit by the methods generally in use the tubes are incubated for one hour and the unit determined on the basis of that time interval; but in the actual serum analysis readings are made at the end of the first hour, the tubes then reincubated and read the end of the second hour, and occasionally the final reading is not made until the following day. Surely this is wrong—for it may easily be proved (cf. Chart No. 3) that in reading a titration set at the end of one hour the unit of complement may be 0.5 c.c.; but if reincubated for an hour it will be seen that hemolysis has proceeded further and that the complement unit may be 0.4 c.c., and if allowed to stand for several hours or over-night the complement unit may be even lower, 0.35 c.c. or 0.3 c.c. This is in keeping with the observation and explains the fact, that sera that are weakly positive when read at the end of one hour may have become negative if read at the end of the second hour—the usual time limit.

CHART III.—DEMONSTRATING THAT THE AMOUNT OF HEMOLYSIS DEPENDS ON THE LENGTH OF INCUBATION. .25 C.C. SHEEP'S CELLS, 1 TO 10, = 7.3 CELL UNITS.

	Tube No.	1	2	3	4	5	6
	Complement 1 to 10, c.c.	.1	.2	.3	.4	.5	.6
	Amboceptor, c.c.	.5	.5	.5	.5	.5	.5
	Sheep's cells 1 to 10, c.c.	.25	.25	.25	.25	.25	.25
	Saline			q. s. 2.5 c.c.			
Unhemolyzed cells, or cell units, after	45 min. incubation	3.8	1.6	.6	.2	0	0
	90 min. incubation	3.1	.7	.2	0	0	0
	135 min. incubation and 6 hrs. at room temperature	2.5	.3	0	0	0	0

The second, the most important error, and the one most difficult to correct, is the element introduced by personal equation in reading the end reactions by the visual method, the one most generally in use. Given two serologists of equal skill, each using the same plan of technique and the same reagents, when the final reading is made, say at the end of the two-hour limit, by the visual method, that is by visually estimating the amount of hemolysis that has taken place and the amount of sheep corpuscles that remain unhemolyzed, one serologist may call this serum weakly positive and the other may call it a delayed negative. In other words, there may be an error approaching 25 per cent. on the part of the one or the other worker and each may think he is right. But it is severe on the clinician, who is sometimes left in doubt, and it is doubly hard on the patient. The error may approach 25 per cent. because it is customary in most laboratories to divide their scale of positiveness into four groups. Serologist A[6] classifies his sera as follows:

++ indicates absolute inhibition of hemolysis or a positive reaction.

+ indicates anything between absolute inhibition and 50 per cent. of inhibition of hemolysis.

± indicates between 50 per cent. of inhibition of hemolysis and total hemolysis, or practically a negative reaction.

− indicates total hemolysis, or a negative reaction.

Serologist B[7] classifies his sera as follows:

1+ or total inhibition of hemolysis.

$\frac{3}{4}$+ or where approximately three-fourths of cells are unhemolyzed.

$\frac{1}{2}$+ or where approximately one-half of cells are unhemolyzed.

$\frac{1}{4}$+ or where approximately one-fourth of cells are unhemolyzed.

0 or complete hemolysis.

Serologist C classifies his sera as follows (our former classification):

+4 or total inhibition of hemolysis.

+3 or where approximately three-fourths of cells remain unhemolyzed.

+2 or where approximately one-half of cells remain unhemolyzed.

+1 or where approximately one-fourth of cells remain unhemolyzed.

— or where complete hemolysis has taken place.

These various degrees of positiveness are then named "very strongly positive," "positive," "positive, moderately strong," "weakly positive," "delayed negative," and "negative or sharply negative."

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[6] Charles F. Craig, Jour. Am. Med. Assoc., April 13, 1914, p. 1232.

[7] John L. Laird, personal communication.

It will be seen that in the scale of Serologists B and C the difference in each degree is relative one of 25 per cent., while in the scale of Serologist A it is nearly 50 per cent.

It is in avoiding such an error of personal equation that the proposed modification has possible merit in providing a uniform standard of reading on an absolute percentage scale.

Certain sera may be sufficiently strong in syphilitic antibodies as to be capable of absorbing more than one unit of complement, so strong that it may not be until three or four full complement units have been added that the "antibody equation" has been satisfied and enough complement in excess has been left to cause total or partial hemolysis (cf. Chart No. 4). These sera, by the proposed method, can be equally well percentaged to two, three, or four hundred "per cent. positive" or fractions thereof, where absorption of each complement unit is equivalent to 100 per cent. By carrying out this procedure in all "100 per cent. positives" the clinician will be informed of the *exact strength* of syphilitic antibody content, and will therefore not be surprised that the Wassermann reaction in a given case does not become reduced more quickly from a "+4" result in the face of urgent treatment, when in reality that serum titrates to a "+8" or a "+12" positive or by our proposed scale "200 per cent. or 300 per cent. positive" or fractions thereof.

CHART IV.—DEMONSTRATING METHOD OF RECORDING RESULTS OF SERA WHICH ABSORB MULTIPLE UNITS OF COMPLEMENT. ALPHA = 17.3. BETA = 4.9.

Tube No.	Patient's serum, c.c.	Complement units.	Antigen units.	Saline, c.c.		Amboceptor, units.	Sheep's cells 1 to 10, c.c.		Cells in front row centrifuge units.	Cells in back row centrifuge units.	Front minus back.	100 per cent.	Result.
1	.1	1	1	q. s. 1.5	Incubate for 45 min. in water-bath at 37.5° C.	1	.5	Incubate for 45 min. in water-bath at 37.5° C.	17.5	4.1	13.4	13.2	100
2	.1	2	1	q. s. 1.5		1	.5		17.5	4.6	12.9	12.7	100
3	.1	3	1	q. s. 1.5		1	.5		17.0	4.2	12.8	13.1	100
4	.1	4	1	q. s. 1.5		1	.5		14.4	4.5	9.9	12.8	77

Result = 377 per cent.

We urge the necessity of using small-bore pipettes throughout the reaction to insure closer accuracy, and we believe that 1 or 2 c.c. pipettes graduated in hundredths should be used for all reagents save possibly the saline. It stands to reason that in a reaction whose delicacy is checked up at so many points (*i. e.*, in the determination of the exact complementary, hemolytic and antigenic units, etc.) it is unwise to risk an error of pipettage by using a 10 c.c. pipette graduated in tenths, when even in the hands of the most expert an occasional error creeps in when filling a hundred or more tubes.

This holds with all the reagents, but most particularly with the sheep's cell emulsion, even in filling a small series of tubes, for the reason that by gravity alone an emulsion of cells in a 10, 5, or even 2 c.c. pipetted will fall toward the tip of the pipette, so that the first unit pipetted out will be richer in cells than will subsequent ones. This error will be minimized by using throughout 1 c.c. pipettes graduated in hundredths. The real extent of this error is not appreciated until one makes use of the centrifuge tubes and by centrifugation determines the difference in cell bulk or cell units (cf. Chart No. 5). Another point in this connection is to use the utmost care in homogenizing the sheep's emulsion by proper and uniform shaking so that each tube shall receive as nearly as possible the same unit of blood. To this end we have discarded the shaking of cells in a flask or graduate and at the time in the reaction when the sheep cells are to be used an assistant pours the emulsion from one beaker to another twenty times before the first pipetteful is drawn and continues such decantation until all the tubes have been filled. All these refinements of technique are time-consuming and laborious, and may be criticized from that viewpoint; nevertheless, we believe that they are essential to obtain the highest degree of accuracy.

CHART V.—DEMONSTRATING THE INCREASE IN ACCURACY IN PIPETTAGE BY USING 1 C.C. PIPETTE INSTEAD OF 10 C.C. PIPETTE.

		Cell Units.						
.5 c.c. sheep's cells, measured with	10 c.c. pipette	20	24	19.9	21	21.2	20.7	21.2
		Greatest difference = 4.1 units.						
	1 c.c. pipette	20.1	20	19.9	19.6	19.7	19.7	19.4
		Greatest difference = 0.7 unit.						

It may be urged against this proposed method that the total time for performance of the reaction will be lengthened. This is not so. On the contrary there is an actual shortening of the total time limit together with an increase in accuracy. The only reason why the two-hour limit is imposed for final readings by the visual method is to permit of the sedimentation by gravity of the unhemolyzed cells, and even at the expiration of this time limit only the completely hemolyzed (or negative) tubes and those showing total inhibition of hemolysis (the positive sera) can be accurately read and the others may have to stand twelve hours or more in the refrigerator before they can be safely estimated by the visual method, and even here the error of personal equation enters. Twenty-five serum reactions may be transferred from Wassermann tubes to centrifuge tubes, centrifuged, read, and recorded in percentage nomenclature within sixty minutes' time. This has been repeatedly checked in our hands and that, too, when we have had only sixteen centrifuge tubes at our disposal.

As regards the cleaning of the capillary end of the tube the difficulty is easily overcome by using a Wright pipette attached to the water faucet by a length of rubber tubing and the stream of water conducted directly into the capillary end of the centrifuge tube. After use the tubes are all thoroughly washed at once, dried in the sterilizer, and before using again are thoorughly rinsed with fresh 0.85 per cent. saline. When properly cared for these tubes are not as fragile as they look, as witness the fact that our sixteen tubes have been in use for weeks without a single tube mortality.

The only valid objection to this method lies, possibly, in the cast of the tubes, our original set costing one dollar each, but we are assured by the glass manufacturers that such tubes, prepared from Jena glass and certified may be bought in bulk for fifty cents each, or possibly less. Sixteen centrifuge tubes will be found sufficient in those laboratories where the number of sera to be tested does not exceed twenty-five.

In passing we wish to put on record the observance of the following phenomenon, namely, that in titrating the complement unit with 0.25 c.c. of 1 to 10 sheep's corpuscle emulsion (which is equivalent to 0.5 c.c. of 1 to 20 emulsion) we may find that 0.5 c.c. is the "compliment unit" (*i. e.*, the smallest amount of complement which, with a given unit of hemolysin, will cause complete hemolysis of 0.25 c.c. of 1 to 10 sheep's cells); we also found that this same amount of complement, namely, the 0.5 c.c. = 1 unit, when used with the same amboceptor unit was capable of hemolyzing almost double the amount of sheep's corpuscles, namely, 0.5 c.c. of 1 to 10 emulsion (cf. Chart No. 6).

CHART VI.—0.5 C.C. SHEEP'S CELLS = 17.2 CELL UNITS. 0.25 C.C. SHEEP'S CELLS = 8.5 CELL UNITS.

Tube No.	1	2	3	4	5	6	7	8
Complement 1 to 10, c.c.	.5	.6	.7	.8	.5	.6	.7	.8
Amboceptor 1 to 2000, c.c.	.5	.5	.5	.5	.5	.5	.5	.5
Sheep's cells, 1 to 10, c.c.	.25	.25	.25	.25	.5	.5	.5	.5
Saline	q. s. 2.5 c.c.							
	Incubated for 45 min. at 37.5 ° C. in water bath.							
Unhemolyzed cells, units	.4	.2	.1	0	4.2	2.9	2.7	2.3
Hemolyzed cells, units .	8.1	8.3	8.4	8.5	13.0	14.3	14.5	15.5